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# Prediction of temporal gene expression

## Metabolic optimization by re-distribution of enzyme activities

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A computational approach is used to analyse temporal gene expression in the context of metabolic regulation. It is based on the assumption that cells developed optimal adaptation strategies to changing environmental conditions. Time-dependent enzyme profiles are calculated which optimize the function of a metabolic pathway under the constraint of limited total enzyme amount. For linear model pathways it is shown that wave-like enzyme profiles are optimal for a rapid substrate turnover. For the central metabolism of yeast cells enzyme profiles are calculated which ensure long-term homeostasis of key metabolites under conditions of a diauxic shift. These enzyme profiles are in close correlation with observed gene expression data. Our results demonstrate that optimality principles help to rationalize observed gene expression profiles.

*Keywords*: evolutionary optimization; mathematical modelling; metabolic regulation; gene expression.

Microarray technologies provide the means to measure simultaneously the expression patterns of thousands of genes [1,2]. These expression data and the availability of more than 80 fully sequenced genomes represent an enormous quantity of experimental data. The conversion of this genomic information into knowledge on phenotype characteristics such as metabolic pathways or signal transduction networks is a challenging task that cannot be effectively tackled without broad application of theoretical and computational methods.

Time resolved tracing of expression levels for large sets of genes has provided evidence that mRNA levels of metabolic enzymes often change within the same time scale as variations of external conditions [1–4]. Quantitative simulation of these time dependent gene expression patterns meets with difficulties due to incomplete knowledge of the underlying regulatory mechanisms. However, statistical methods have been successfully applied, such as cluster analysis of time-dependent gene expression patterns for identifying functionally related proteins [5–10].

It has been stressed that even without detailed knowledge of gene regulatory mechanisms phenotype properties can be rationalized by evolutionary optimization principles [11]. The basis of this approach is the hypothesis that a permanent change of phenotype properties due to mutation and selection leads to an optimal adaptation of an organism to given environmental conditions. Most optimization studies in the field of metabolic regulation are aimed at prediction of time independent characteristics of enzymes ensuring optimal performance of metabolic pathways [11–15]. The microarray data suggests applying optimization concepts to also explain time courses of enzyme concentrations.

The basic idea of our paper is that time dependent gene expression enables cells to adapt their metabolic capabilities in an optimal way to varying external conditions. Our approach consists in (a) establishing a mathematical model of the metabolic pathways under consideration, (b) defining a performance function to evaluate in a quantitative manner the functioning of the cell under given external conditions, (c) calculating time-dependent enzyme concentration profiles (henceforth called enzyme profiles) which optimize the performance function, and (d) comparing the predicted optimal enzyme profiles with experimental expression data.

Optimization of the network is performed under the constraint that the total available enzyme concentration is limited by the protein synthesizing capacity of a cell [16]. The optimization problem thus consists in distributing in a time-dependent manner a finite amount of protein to the participating enzymes. As a consequence, an increase in the concentration of one enzyme must be compensated to a certain extent by the decrease in the concentrations of other enzymes.

As a first instructive example we deal with a linear chain of monomolecular enzymatic reactions. We address the question how the concentrations of the enzymes have to vary in time to accomplish a fast conversion of the initial substrate into the final product. Next, we analyse gene regulation of a complex metabolic system, the central metabolism of *Saccharomyces cerevisiae* under conditions of the diauxic shift. For this case time dependent gene expression data are available [1]. We measure the metabolic performance in terms of the survival time at glucose

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*Enzymes*: 6-phosphofructokinase (EC 2.7.1.11); hexokinase (EC 2.7.1.1); glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12); enolase (EC 4.2.1.11); alcohol dehydrogenase (EC 1.1.1.1); pyruvate decarboxylase isozyme 1, 2, and 3 (EC 4.1.1.1); aldehyde dehydrogenase (NAD(P) +) (EC 1.2.1.5); acetyl-CoA synthetase (EC 6.2.1.1); isocitrate dehydrogenase (NAD +) subunit 1 and 2 (EC 1.1.1.41); fumarate dehydrogenase (EC 4.2.1.2).

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starvation and predict optimal enzyme profiles of various metabolic pathways.

## RESULTS

### Temporal waves in enzyme profiles for unbranched pathways

Scheme 1 in the Appendix shows an idealized unbranched model pathway consisting of $n$ consecutive enzyme-catalyzed monomolecular enzymatic reactions and a series of $n - 1$ intermediates, $X_i$. We assume that the product $P$ represents a biochemical compound whose availability is rate-limiting for the reproduction of an individual: the faster the substrate $S$ can be converted into this product, the more efficient the individual may reproduce and out-compete other individuals. As a measure of the average time to produce $P$ from $S$ we use the transition time $\tau$ as defined in [17] (see also legend to Fig. 1). The optimization problem to be solved reads $\tau = \min$ at the constraint that the total available enzyme concentration may not exceed an upper bound $E_{tot}$, i.e. $\Sigma E_i \leq E_{tot}$. The metabolic process is initiated by addition of substrate to an 'empty' pathway, i.e. except S all metabolites have zero concentrations at the beginning.

For the simplest case $n = 2$, an explicit solution can be found for the optimization problem (see legend to Fig. 1; derivation of the analytical solution for the two-component linear reaction chain is available from the authors on request). The optimal enzyme profiles and related metabolite concentrations shown in Fig. 1 comprise two phases separated by a single switch at time $t = T_1$. During the initial phase, $t < T_1$, the whole amount of protein is allocated to the first reaction ($E_1 = E_{\text{tot}}$, $E = 0$). At the beginning of the second phase the concentration $E_2$ undergoes an abrupt switch from zero to a finite value whereas the concentration $E_1$ is decreased by the same extent.

An intriguing finding is that the final product is produced only in the second phase, i.e. paradoxically the fastest possible conversion of the substrate into the final product is achieved with a delayed onset in the formation of P. The optimal enzyme profile depends on the choice of the initial concentrations of the metabolites. If, for example, the initial ratio $r = X_1/S$ exceeds the threshold value $r_{\text{crit}}$ given by the ratio $E_2/E_1$ in the second phase of the solution shown in Fig. 1, the optimal enzyme profiles are still given by a single abrupt switch at time T1 but now in the first phase of the process the whole amount of enzyme is allocated to the second enzyme instead to the first one. r affects only the value the switching time $T_1$ but not the ratio $E_2/E_1$ in the second phase of the process [18]. The initial refrain from spending protein to the second reaction and thus from synthesizing P at the beginning pays off in the later stage of the process.

For longer pathways, $n > 2$, the optimization problem was solved numerically. The unknown enzyme profiles, $E_i(t)$, were approximated by a stepwise constant function, i.e. the whole time axis was subdivided into a fixed number of time intervals and the enzyme concentration was put to constant values within these time intervals. The quantities to be optimized are the switching times $T_1$, $T_2$, etc. defining the time intervals and the constant enzyme concentrations between the switching times; for details of the nonlinear minimization procedure, see legend to Fig. 2. In these calculations the number $m$ of allowed switches was successively increased, starting with $m = 0$. At an arbitrary but fixed number of switches, the switching times and the constant enzyme levels within the time intervals were

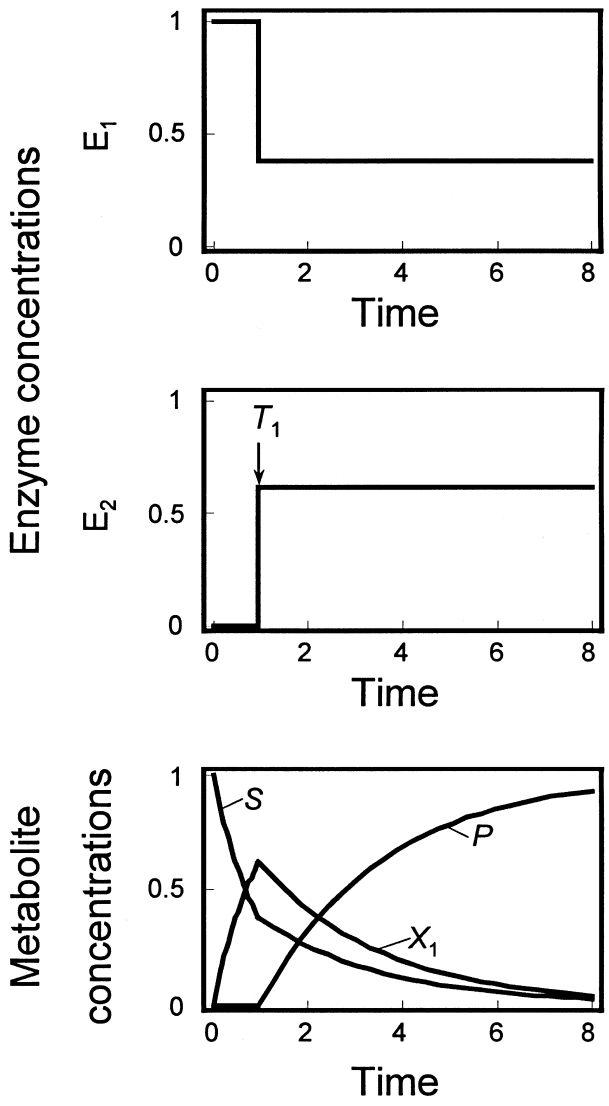



**Fig. 1. Optimal enzyme profiles and metabolite time courses for the linear metabolic pathway ($n = 2$).** The mathematical description was based on linear kinetic equations presented in the Appendix. Optimization was performed under the constraint $\Sigma E_i \leq E_{\text{tot}}$ where $E_{\text{tot}}$ represents a fixed total concentration of enzymes. The performance function to be minimized is the transition time $\tau$ needed to convert the substrate into the product [17]. $C$ denotes the initial concentration of the substrate and equals at any time point the total metabolite concentration in the system, i.e. $C = S + P + \sum_{i=1}^{n-1} X_i = const$. Calculations were performed for equal catalytic efficiencies of the enzymes ($k_i = k$). The analytical solution of the optimization problem reads [18]: $\Sigma E_i \leq E_{\text{tot}}$, i.e. the maximum available amount of protein is actually used; switching time $T_1 = \ln(2/(3-\sqrt{5}))$. First time interval ($t \geq T_1$): $E_1 = 1, E_2 = 0$; second time interval ($t > T_1$): $E_1 = (3-\sqrt{5})/2$, $E_2 = \ln(\sqrt{5}-1)/2$. Optimal transition time, $\tau_{\min} = 1 + T_1 + (1-e^{-T_1})^{-1} = 3.58$. Enzyme concentrations are given in units of $E_{\text{tot}}$; times are given in units of $(k \cdot E_{\text{tot}})^{-1}$.

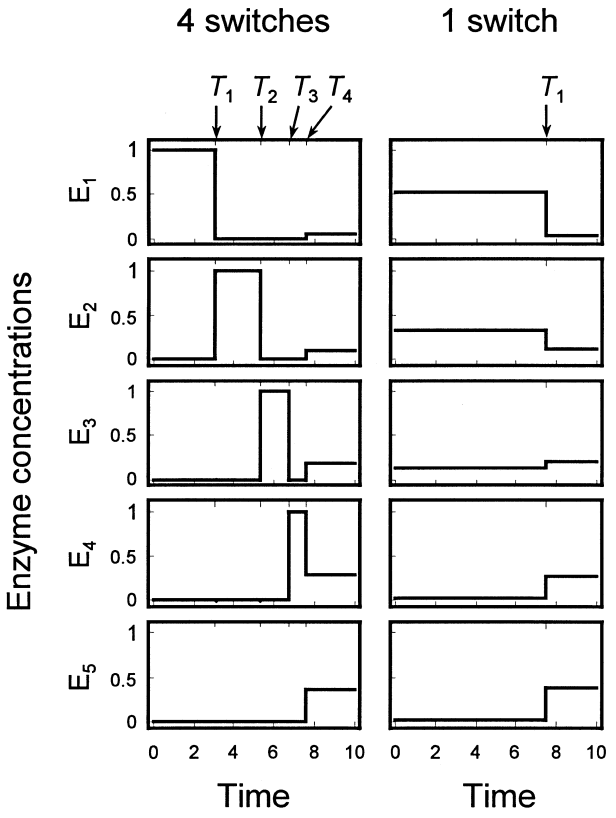



**Fig. 2. Optimal enzyme profiles and metabolite courses for the linear metabolic pathway ($n = 5$).** Column 1: optimal enzyme profiles yielding the absolute minimum of the transition time (four switches, $m = 4$): $T_1 = 3.08$, $T_2 = 5.28$, $T_3 = 6.77$, $T_4 = 7.58$. Column 2: optimal enzyme profiles yielding minimum of the transition time if only a single switch is allowed ($m = 1$): $T_1 = 7.45$. Calculation procedure: the time axis was divided into $m+1$ intervals: $T_{j-1} \leq t < T_j$ (whereby $T_0 = 0$ and $T_{m+1} \rightarrow \infty$). Within each time interval $j$ the enzyme concentrations $E_i(j)$, $i = 1,\ldots,n$, are constant. The enzyme concentrations may switch to new values between two intervals. Optimization involves the following steps: (1) explicit solution of the system equations for $P(t)$ as a function of the $m$ unknown switching times $T_j$ and the $(m+1) \times n$ unknown enzyme concentrations $E_i(j)$ (2) explicit calculation of the transition time $\tau$ and (3) minimization of $\tau$ by a steepest descent method leading to optimal values of $T_j$ and $E_i(j)$. Initial conditions at $t = 0$: $S = C\ X_i = P = 0$.

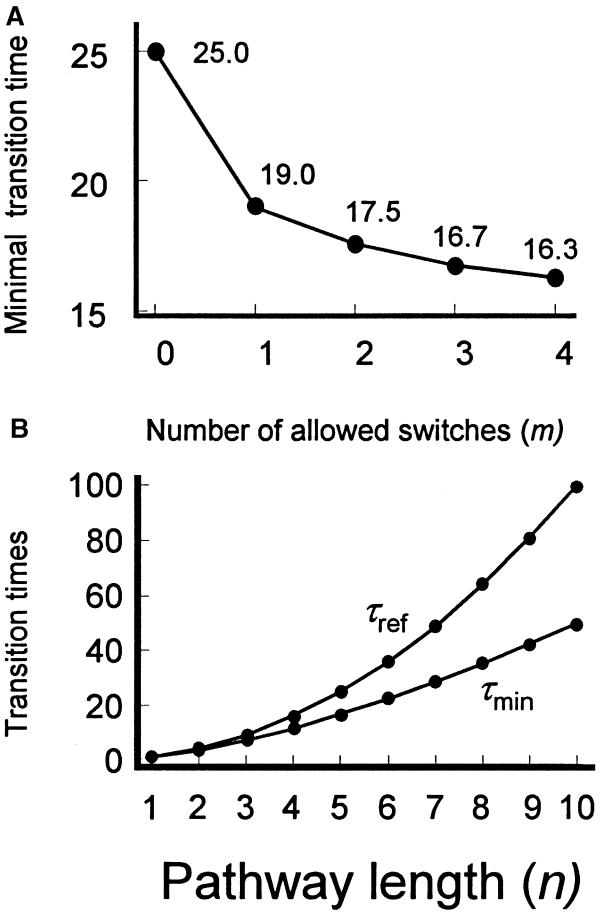



**Fig. 3. Minimal transition times.** (A) Minimal transition time for the linear metabolic pathway ($n = 5$) as function of the number $m$ of allowed switches of the enzyme concentrations. The minimal transition time obtainable with time-dependent enzyme profiles was calculated as outlined in Fig. 2. The largest drop in the transition time (from 25 to 19 time units) is already achieved by allowing for a single switch ($m = 1$). The absolute minimum of the transition time is achieved by four switches, i.e. a higher number of switches does not lead to a further decline of the transition time. (B) Minimal transition times at varying length of the linear metabolic pathway. The minimal transition time $\tau_{min}$ obtainable with time-dependent enzyme profiles was calculated as outlined in Fig. 2. In order to demonstrate the advantage of metabolic regulation of time-dependent enzyme expression the reference value $\tau_{ref}$ is also shown representing the minimal transition time obtainable at time-independent enzyme concentrations.

determined such that the transition time became a minimum. Figure 3A depicts how the minimal transition time decreases with increasing number of switches for a linear reaction chain of length $n = 5$. Interestingly, a major reduction of the transition time is already brought about if a single switch in the enzyme concentrations occurs at an appropriate time. The corresponding enzyme profiles are shown in the second column of Fig. 2.

The optimization procedure was stopped when a further increase in the number of allowed switches did not lead to a further decrease of the transition time $\tau$ . For the linear reaction chain of length $n = 5$ the absolute minimum of the transition time was obtained by allowing for $m = 4$ switches. The corresponding optimal enzyme profiles are shown in the first column to Fig. 2. These optimal enzyme profiles have the following characteristics: Within any time interval, except of the last one, only a single enzyme is fully active whereas all others are shut off. At the beginning of the process, the whole amount of available protein is spent exclusively to the first enzyme of the chain. Each of the following switches turns off the active enzyme and allocates the total available protein to the enzyme catalysing the following reaction. The last switch allocates a finite fraction of protein to all enzymes whereby the first enzyme of the chain (which has already done most of its 'work' in converting S into $X_1$) takes the smallest share and the last reaction (which yet has to do most of its 'work' in converting $X_4$ into P) takes the largest share. The optimal allocation of protein to the various enzymes resembles a 'soliton-like' wave which propagates through the reaction chain in such a manner that the highest expression of an enzyme takes place

just at the right time to ensure efficient conversion of its accumulated substrate.

Similar calculations performed for longer and shorter pathways have shown that the transition time always attains the absolute minimum when the number of switches is one less than the number of reactions, i.e. $m = n - 1$; allowing for more switches yielded no further decrease in the transition time. The optimal enzyme profiles had always the above outlined wave-like characteristics with the peculiarity that within the last time interval the available protein is spread over all reactions to ensure complete conversion of the initial substrate into the end product.

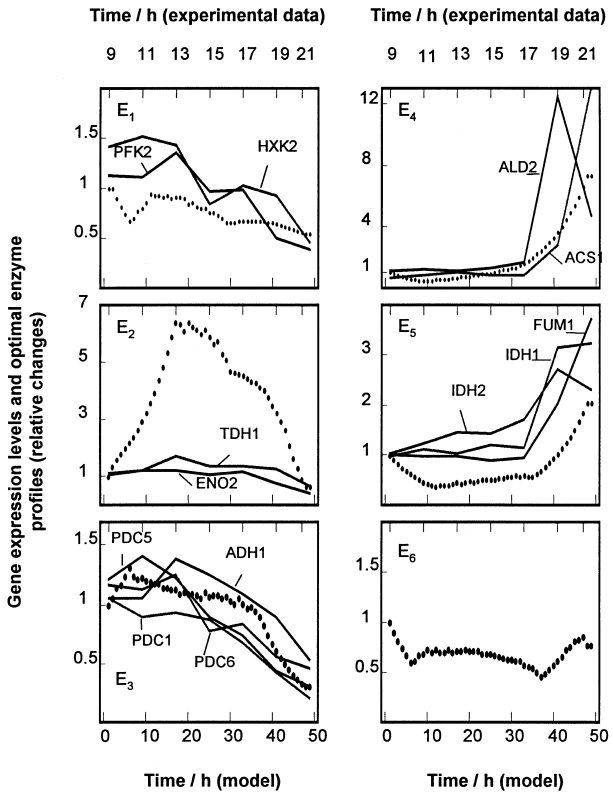
**Fig. 4. Optimal of enzyme profiles ensuring maximal survival time of yeast cells under conditions of a diauxic shift.** Optimal enzyme profiles (dotted curves) were calculated for Scheme 2 and governed by the kinetic equations given in the Appendix. Related observed gene expression profiles are plotted as solid curves. Rate constants: $k_1 = 3.7$, $k_2 = 6{\cdot}10^3$, $k_3 = k_4 = 10^4$, $k_5 = k_6 = 4{\cdot}10^3$, $k_7 = 1.28$, $k_8 = k_9 = 12$. In the feeding period ($t \leq 0$) the system was assumed to be in steady state characterized by the glucose influx $v_0 = 9.96$, the metabolite concentrations $X_1 = 5.8$, $X_2 = 0.9$, $X_3 = 0.2$, $X_4 = 8.7$, $NADH = 0.1$, $ATP = 2.4$, and the enzyme concentrations $E_1 = 0.1934$, $E_2 = 0.0909$, $E_3 = 3.0621$, $E_4 = 0.0078$, $E_5 = 0.9208$, $E_6 = 1.7250$. Time given in h, concentrations given in mM. Calculations were performed for the threshold values $ATP_{\min} = 1.55$ and $NADH_{\min} = 0.05$. At $t = 0$ the stationary feeding period was stopped by preventing the further supply of glucose ($v_0 = 0$ for $t > 0$). The concentrations of metabolites and enzymes of the feeding period were taken as initial values for the starvation period. The time-dependent enzyme profiles were approximated by interval-wise constant values, i.e. $E_i(t) = E_i(j)$ for $T_j \leq T_{j+1}$ between equidistantly distributed time points, $T_j = j$, with $j = 0,\ldots,m$ ($m$: number of switches). Maximization of the survival time by means of a genetic algorithm: A population was introduced as a set of species $S^p$ each characterized by the survival time $\vartheta(p)$ associated with a given set of $E_i^p(j)$ values in the time intervals $j$ with $\sum_{i=1}^{6} E_i^p(j) \leq E_{\text{tot}}$. The optimization procedure started with a randomly chosen population. This population was subjected to a certain number of mutations and recombination's. A 'mutation' is defined as exchange of a small amount ($\delta E$) of protein between randomly chosen enzymes $E_i$ and $E_{i^*}$ taking place in a randomly chosen time-interval $j$, i.e. $E_i(j) \rightarrow E_i(j)$–$\delta E_i$, $E_{i^*}(j) \rightarrow E_{i^*}(j)$–$\delta E_{i^*}$ ($i \neq i^*$). $\delta E_i$ and $\delta E_{i^*}$ have to be consistent with the constraint of an upper limit for the sum of all enzyme concentrations. To prevent irregular enzyme profiles the maximum possible change in the concentration of a given enzyme between two succeeding time intervals was restricted to 10%. Recombination is defined as exchange of all values of $E_i(j)$ between two randomly chosen species for all time-intervals $j \geq j_0$ with a randomly chosen $j_0$. A new population of species was selected after a sufficiently large number of mutations and recombination's whereby the probability of a species to enter the new population was proportional to the value of the survival time $\vartheta(p)$. Comparison with measured gene expression profiles: for comparison to experimental data expression data of those genes which belong to the group represented by overall reactions 1–6 are displayed as solid lines represent the red-over-green fluorescence ratios ('fold induction/repression') picked up from the Stanford Microarray database (http://cmgm.stanford.edu/pbrown/explore/array.txt). For $E_1$ (upper glycolysis): HXK2 and PFK1, for $E_2$ (lower glycolysis): TDH1 and ENO2, for $E_3$ (ethanol formation): PDC1,5,6 and ADH1, for $E_4$ (ethanol degradation): ALD2 and ACS1, and for $E_5$ (TCA cycle): IDH1,2 and FUM1. Time scales of experimental data (shown above the panels) and model predictions (shown below the panels) differ by a factor of about 2.

The gain in 'functional efficiency' accomplished by optimal time dependent variations of enzyme concentrations was assessed by comparing the minimal transition time $\tau_{\min}$ with the reference value $\tau_{\text{ref}}$ representing the smallest possible transition time achievable without time dependent enzyme variations (Fig. 3B). As shown in [19] the transition time at constant enzyme concentrations is minimized when equal amounts of protein are allocated to all enzymes, i.e. $E_i = E_{\text{tot}}/n$ (giving rise to the functional dependency $\tau_{\text{ref}} \propto n^2$). It is seen that the difference between $\tau_{\min}$ and $\tau_{\text{ref}}$ due to time dependent optimization of enzyme profiles steadily rises with increasing length of the pathway (e.g. 10.5% for $n = 2$ and 50.2% for $n = 10$).

## Predicting temporal enzyme profiles for central metabolic pathways of yeast cells under conditions of a diauxic shift

Using microarray techniques it was discovered that the switch from fermentation to respiration after depletion of glucose is accompanied by concerted changes in the mRNA levels for most enzymes of the central metabolism of yeast resulting in down-regulation of glycolysis and up-regulation of the TCA-cycle and gluconeogenesis [1,3]. In this paragraph we report on the application of our optimization approach to rationally explain these observed time dependent changes as a strategy of yeast cells to maintain the concentration level of important metabolites. The starting point is the simplified metabolic governed by the kinetic equations given in the Appendix.

The diauxic shift is a peculiarity of yeast cells to utilize ethanol under conditions of glucose depletion to maintain their cellular redox potential NADH/NAD and ATP level.



This enables them to survive over longer periods of starvation. Accordingly, we have chosen as performance function the 'survival time', $\vartheta$, defined as the time span during which the redox potential and energetic status of the cell represented by the concentrations of the key substances NADH and ATP, remain above critical thresholds.

Optimal enzyme profiles were calculated by maximizing $\vartheta$ under the constraint that the sum of individual enzyme concentrations during the time course must not exceed the total initial enzyme concentration. For $t < 0$ (feeding period) we assumed time-independent concentrations of enzymes such that the steady state solutions of the model equations yield metabolites concentrations and fluxes which are consistent with reported values [20]. The starvation period was initialized at time $t = 0$ by interrupting the supply of glucose ($v_0 = 0$ for $t \geq 0$). Calculation of optimal enzyme profiles was performed by using a similar discretization technique as applied to the search of optimal solutions for the unbranched pathways. The time axis was subdivided into a large number of time-intervals off equal lengths $\Delta t = 1$. The search for optimal values of the unknown enzyme concentrations within each time-interval was carried out by means of a genetic algorithm [21] detailed in the legend to Fig. 4.

The obtained optimal enzyme profiles are shown in Fig. 4 (dotted curves). The related time-dependent concentration courses for the metabolites NADH, ATP and ethanol are depicted in Fig. 5 (curves a). For comparison, Fig. 5 also shows the optimal concentration courses for cases where only a single switch of the enzyme activities was allowed (case b) or no switch was allowed at all (case c).

Inspection of the enzyme profiles in Fig. 4 reveals that initiation of the starvation period gives rise to a notable initial increase in the activity of the lower part of glycolysis ($E_2$). This effect is paralleled by an increase in the activity of ethanol formation ($E_3$). Hence, as long as glucose is not exhausted it is advantageous for the cell to direct glycolysis to the replenishment of the ethanol reservoir to make use of it in a later phase of starvation. Increasing activity in the lower part of glycolysis ($E_2$) enhances the consumption of triose-phosphates and thus causes a rapid switch-off of the synthetic pathway (reaction 9). The model predicts non-monotonic profiles for the enzymes of the TCA cycle ($E_5$) and of aerobic ATP production ($E_6$). An initial decrease is followed by a plateau before a final increase. In the later phase of the starvation period, when the glycolytic metabolites are exhausted, the lower part of glycolysis ($E_2$) and the ethanol forming reactions ($E_3$) are switched off. This allows to allocate the available amount of protein to the ethanol utilizing enzymes ($E_4$) making the ethanol pool available for the formation of NADH. Accordingly, there is a strong increase in the activity of the tricarbonic acid cycle ($E_5$) and the respiratory chain ($E_6$) to compensate for the decline in the glycolytic supply of NADH and ATP.

For a comparison to experimental results we display in Fig. 4 the time dependent expression profiles of several genes ([1], http://cmgm.stanford.edu/pbrown/explore/array.txt) which are related to the groups of the enzymes entering Scheme 2 in the Appendix. There is a remarkable concordance of the predicted enzyme profiles and observed gene expression profiles. In all cases the tendencies (increase or decrease) are correctly predicted by the model. In particular, the 'fold increase/decrease', i.e. the ratio between the final and the initial expression level, match very well.

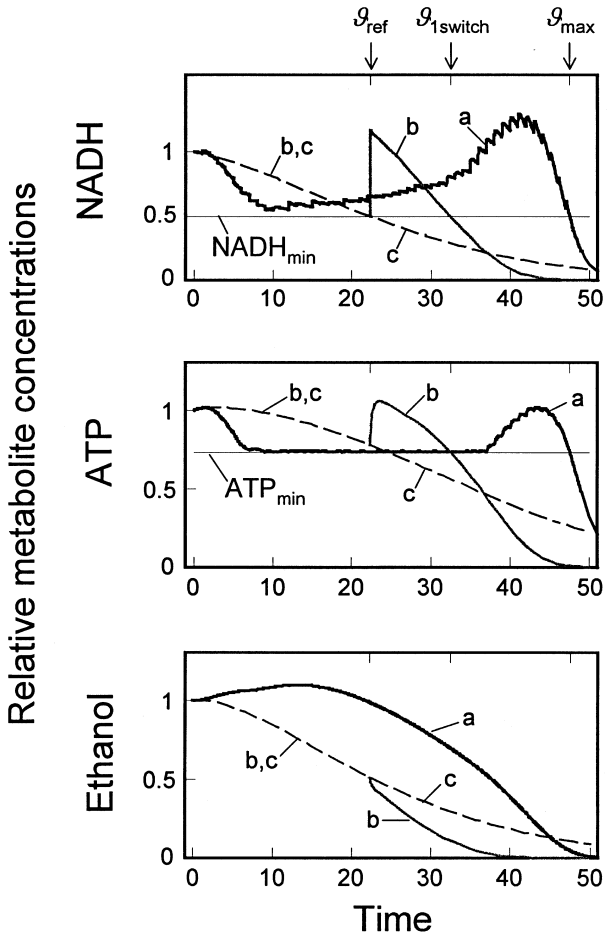



**Fig. 5. Calculated time-courses of some important metabolites of yeast at enzyme profiles ensuring a maximal survival time of yeast cells.** The concentrations of NADH, ATP, and ethanol are represented as relative values with respect to their initial values. (a) Time courses correspond to the optimal enzyme profiles depicted in Fig. 4. (b) Time courses correspond to a optimal single switch of enzyme activities. (c) Time courses at time-independent enzyme profiles.The vertical arrows above the upper panel indicate the maximal survival times achieved in cases (a–c). Thin horizontal lines in the upper two panels indicate the threshold values for NADH and ATP, respectively.

The time courses of the metabolite concentrations in Fig. 5 indicate that reprogramming of gene expression under stress conditions allows for homeostasis of metabolites as NADH and ATP which are essential for cell viability. The calculated survival time amounts to $\vartheta_{max} = 47.55$ (see curves a) which is about twice as large as the survival time $\vartheta_{ref} = 22.32$ obtained for time-independent enzyme concentrations (see curves c). At the respective $\vartheta$ values the concentration of either NADH or ATP fall below their thresholds. It is intriguing that even a single switch in the enzyme carried out at an optimal time point leads to a pronounced prolongation of the survival time ($\vartheta_{1\ switch} = 32.94$, curves b in Fig. 5).

## DISCUSSION

In this paper, we have applied optimality principles to rationalize time-dependent gene expression profiles in the

context of cellular metabolism. In its mathematical foundation our approach shares a lot of similarities with methods applied in the theory of optimal control [22]. From the biological view point, our approach is backed up by many observations pointing to the existence of time-dependent gene expression patterns which have evolved during natural evolution to assure survival of the population in typical and recurrent stress situations such as shortage of substrates or changes of pH or temperature. We think that such evolutionary trained gene expression patterns represent a sort of 'population memory' that enables cells to cope with environmental changes in an anticipatory way. It has to be noted that the optimization of long-term responses considered in our approach differs from other theoretical approaches in that field considering the maximization of the flux rate through a metabolic pathway at any time as a (short-term) goal of genetic regulation [23,24].

Dealing with the evolutionary optimization of gene expression in mathematical terms requires substantial simplifications in view of the complexity of cellular metabolism. Therefore, the presented work is primarily intended to gather deeper insight into general strategies underlying commonly erratic temporary gene expression patterns rather than to provide a computer tool to exactly predict the expression profile for a specific enzyme. A major simplification of our approach is the restriction to the analysis of relatively small metabolic schemes governed by simple first or second order rate equations. Moreover, only a single performance function (transition time for the conversion of a substrate into a final product, homeostasis of cardinal metabolites) was introduced to measure the fidelity of a metabolic system. Optimal enzyme profiles were calculated under the premise that the optimum of the chosen performance function has been already attained. Finally, the calculated optimal enzyme profiles do not take into account that the redistribution of enzyme within the pathway requires a finite time span due to protein synthesis and degradation. Regarding the latter aspect, we have also analysed extended versions of the unbranched pathway model by including in some detail transcription of genes, translation of mRNAs, and proteolysis. In these models the genes may exist in 'On' or 'Off'-states and it was assumed that mRNAs and enzymes compete for their building blocks (nucleotides and amino acids during transcription and translation, respectively) which occur in finite amounts. Using again the transition time as performance function the optimal solution is characterized by abrupt switches in gene activities which result, however, in smoother variations of the enzyme concentrations. For the limiting case of very fast enzyme turnover the optimal time-dependent enzyme concentrations tend towards the profiles obtained without explicit consideration of enzyme synthesis and degradation.

Our results derived for some model systems underline the common view that temporal gene expression is a powerful means of cells to adjust their metabolism to changing environmental conditions. Turning on or off enzyme activities at appropriate time points may lead to a significant improvement of metabolic efficiency. For the linear reaction pathway of length $n = 10$ the transition time achieved by optimal time-dependent enzyme profiles dropped down to about 50% of the value obtainable at optimal but time-independent allocation of protein to the various enzymes. In case of yeast metabolism, the survival time approximately doubled due to time-dependent regulation of enzyme activities. Considering the huge number of different enzymatic reactions in a cell and the possibility to switch on or off complete pathways the gain in functional efficiency associated with temporal gene expression will possibly be even higher than estimated for the relatively simple metabolic systems studied in this paper. Interestingly, a pronounced impact on the functional efficiency of the metabolic systems studied was already achieved by a single switch in the enzyme concentrations provided that this switch takes place with the right intensity and at the right time. Our theoretical findings suggest that an even better metabolic adaptation to environmental changes should be possible by multiple switching giving rise to nonmonotonic enzyme profiles.

The general inference of our theoretical study is that the limited resources force the cell to concentrate protein synthesizing capacities to those enzymes which are currently needed. This becomes most apparent in the wave-like enzyme profiles for the linear pathway but is also reflected by optimal enzyme profiles in the yeast model. Our results well agree with experimental data. Studies of gene expression during the cell cycle of *Caulobacter crescentus* lead to the conclusion that 'genes involved in a given cell function are activated at the time of execution of that function' [25]. Clustered expression profiles show wave-like temporal changes of mRNA levels [25]. Their findings are supported by proteomic analyses [26].

Our results suggest that an optimal strategy to reach a long-term goal by temporal gene expression is not optimal from the view point of short-time behaviour. In the case of a linear chain this becomes apparent by a lag phase before starting to synthesize the final product. For yeast metabolism global optimization of the survival time is achieved by intermediary storage of ethanol which on a shorter time scale would appear as a waste of glucose. Obviously, such strategies could only be established as a result of an evolutionary process.

As demonstrated for the metabolism of yeast cells our method even allows to predict groups of enzymes which should be coexpressed or differentially expressed under given external conditions. It turns out that the enzymes of one and the same pathway may differ in their individual time profiles (see deviating regulation of upper and lower glycolysis in the initial phase of the diauxic shift, Fig. 4). Similarly, enzymes with synchronized expression profiles may belong to different metabolic pathways. The predictions could be refined by considering more detailed metabolic reaction schemes taken, for example, from the KEGG database of metabolic pathways (http://www.genome.ad.jp/kegg/metabolism.html). In this way our approach may contribute to assign gene expression profiles to enzymes involved in defined parts of metabolism. Our future work will aim at studying whether the proposed methodology can be generalized to more complex, branched metabolic processes, especially in view of predicting expression of the genes most critical to a given process.

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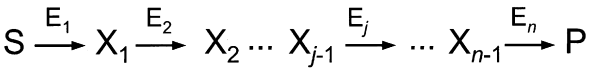
## APPENDIX

### Reaction scheme 1




**Scheme 1. A linear (unbranched) reaction chain of (n) reactions steps converting substrate S into product P.**

Systems equations

$$\frac{dS}{dt} = -k_1 \cdot E_1 \cdot S$$

$$\frac{dX_i}{dt} = k_i \cdot E_i \cdot X_{i-1} - k_{i+1} \cdot E_{i+1} \cdot X_i$$

$$\frac{dP}{dt} = k_n \cdot E_n \cdot X_{n-1}$$

Constraint

$$\sum_{i=1}^{n} E_i(t) = E_{\text{tot}}$$

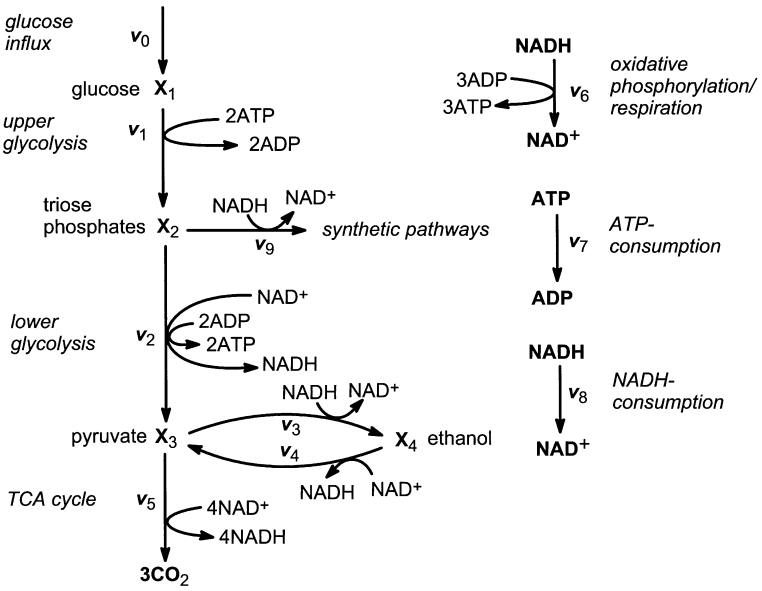
Performance

$$\tau = \frac{1}{C}\int_0^{\infty} (C - P(t))dt$$

$$C = S|_{t=0}$$

$$\tau \rightarrow MINIMUM$$

**Reaction scheme 2**




**Scheme 2. Skeleton model of the central metabolism of yeast.** Groups of enzymes constituting pathways or functional parts of pathways are represented as single overall reactions.

Systems equations

$$dX_1/dt = v_0 - v_1$$
$$dX_2/dt = 2v_1 - v_2 - v_9$$
$$dX_3/dt = v_2 - v_3 + v_4 - v_5$$
$$dX_4/dt = v_3 - v_4$$
$$dNADH/dt = v_2 - v_3 + v_4 + 4v_5 - v_6 - v_8 - v_9$$
$$dATP/dt = -2v_1 + 2v_2 + 3v_6 - v_7$$

$$v_1 = E_1 \cdot k_1 \cdot X_1 \cdot ATP$$
$$v_2 = E_2 \cdot k_2 \cdot X_2 \cdot NAD^+ \cdot ADP$$
$$v_3 = E_3 \cdot k_3 \cdot X_3 \cdot NADH$$
$$v_4 = E_4 \cdot k_4 \cdot X_4 \cdot NAD^+$$
$$v_5 = E_5 \cdot k_5 \cdot X_3 \cdot NAD^+$$
$$v_6 = E_6 \cdot k_6 \cdot NADH \cdot ADP$$
$$v_7 = k_7 \cdot ATP$$
$$v_8 = k_8 \cdot NADH$$
$$v_9 = k_9 \cdot X_2 \cdot NADH$$

Constraint

$$NADH + NAD^+ = const.$$
$$ATP + ADP = const.$$
$$\sum_{i=1}^{6} E_i(t) \le E_{tot}$$

Performance

$$\vartheta = t\ \Theta(ATP - ATP_c)\ \Theta(NADH - NADH_c)$$
$$\Theta(x) = 1 \text{ if } x \ge 0,\ \Theta(x) = 0 \text{ if } x < 0$$
$$\vartheta \rightarrow \text{MAXIMUM}$$